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DETERMINATION OF SINGLE-PORE CONDUCTANCE FROM NOISE ANALYSIS

INFLUENCE OF DISTRIBUTION IN PORE-AMPLITUDES

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Summary

For pores which can adopt only the open and closed states, the influence of the amplitude distribution of the single-pore conductance (γ, open state) on the covariance function is derived. It is shown that reliable mean values of γ ($E(\gamma)$) can be derived from noise analysis only if the variance in the amplitude distribution (σ_γ^2) is known. In the past, σ_γ^2 was set always to zero, leading to an overestimation of $E(\gamma)$. In the case of gramicidin-doped lipid bilayer membranes, this overestimation amounts to as much as 15% of the true value of $E(\gamma)$.

Introduction

Noise analysis of fluctuations in membrane conductance has been widely used to obtain information about the molecular properties of ion-transport mechanisms in biological and artificial membranes [1—7]. Due to the fluctuation-dissipation theorem [8], macroscopic relaxation experiments yield the same information about the kinetic parameters of the underlying molecular events as does the corresponding noise analysis. The mean advantage of the noise analysis is considered to be the information it provides about the amplitude of the underlying elementary conductance process derived from the measured noise intensities. At steady-state, from measurements of the variance of conductance fluctuations generated by the opening and closing of pores and a simultaneous determination of the mean membrane conductance, the mean single-pore conductance may be estimated [1—5]. In this application of noise analysis, the intuitive assumption has always been made that all of the pores adopt an identical value of pore conductance. But, from single-pore experiments on lipid bilayer membranes using gramicidin as a pore-forming substance, it is known that single-pore conductances show a probability distribution with a variance different from zero [8--10].

In this paper we derive from the non-equilibrium steady-state the covariance function of conductance fluctuations of pores, whereby the transition of the pore between the open and closed states is described by a unimolecular chemical reaction. The amplitudes of single-pore conductance may assume values between zero and infinity. Using known probability distributions of single-pore conductances measured for gramicidin-doped bilayer membranes, it will be shown that neglecting the variance in the amplitudes of single-pore conductance always leads to an overestimation of mean single-pore conductance derived from noise analysis.

Theory

In the following, the basic notations will be introduced which are used for the derivation of the covariance function of the compound random process of conductance fluctuations as shown in Fig. 1. The process can be considered as a generalized random-telegraph wave of random amplitude. It will be described by the random variable, $X_t(\omega)$, taking values in the set, $\mathcal{R}_+ = [0, \infty)$. If $b \in \mathcal{R}_+$ the set of all possible outcomes ω (realizations of the fluctuation process), for which at time t the relationship $X_t(\omega) \leqslant b$ holds, is an event, namely, the event $\{\omega : X_t(\omega) \leqslant b\}$, we will write $\{X(t) \leqslant b\}$ for brevity and denote the corresponding probability measure of the event by $P\{X(t) \leqslant b\}$. A particular function which will often be used in the following is the indicator function, $l_B(\omega)$, defined for $B \subset \mathcal{R}_+$ a subset of $\mathcal{R}_+$. $l_B(\omega)$ is a random variable which is equal to 1 if the event $\{\omega \in B\}$ occurs and is equal to 0 otherwise [11]. For brevity we will write l_B instead of $l_B(\omega)$.

The covariance function of the stationary fluctuation-process described by

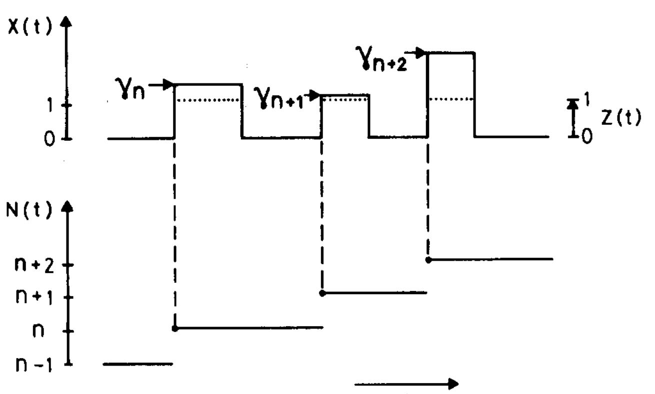

Fig. 1. Sample function $X(t)$ of single-pore conductance fluctuations generated by the opening and closing of pores. The dotted line shows the sample function $Z(t)$ of a generalized random telegraph wave. The single-pore conductances are described by the random variable $\gamma_{(N(t))}$ where $N(t)$ is the counting process associated with the random process.

$X(t)$ is defined by the relationship [11]:

$$C_X(\tau) = E(X(t) \cdot X(t+\tau)) - (E(X(t)))^2 \tag{1}$$

where E denotes the mean of the expression following in parentheses. Three basic assumptions are made for the calculation of $C_X(\tau)$.

First, the pulses are considered to be independent of each other. Second, the on- and off-times of the pulses are exponentially distributed, whereby the parameter, $1/\lambda$, denotes the mean life-time of a pulse and $1/\mu$ the mean off-time between two consecutive pulses. Third, the amplitude of a pulse is considered to be independent of the life-time of the pulse and constant within the life-time of a pulse. This type of dependence is considered elsewhere (Guth, W. and Kolb, H.-A., unpublished results). The random process (see Fig. 1) can be described by the product of the random variable, $Z(t)$, which accounts for a generalized random-telegraph wave of pulse height 1 (see dotted curve in Fig. 1) and the random variable, $\gamma(N(t))$, which has the dimension of a conductance:

$$X(t) = Z(t) \cdot \gamma(N(t)) \tag{2}$$

For all pulses, $Z(t)$ takes the values:

$$Z(t) = \begin{cases} 1 \text{ for pore open at time } t \\ 0 \text{ for pore closed at time } t \end{cases} \tag{3}$$

$\gamma(N(t))$ takes values in $\mathcal{R}_+ = [0, \infty)$ and describes the height of the pulse numbered by $N(t)$. For each outcome of the random wave, $N(t)$ is the number of pulses in the time interval $(0, t]$ (see Fig. 1). For each $t \in \mathcal{R}_+$, $N(t)$ is a random variable which denotes a counting process taking values in the set $\{0, 1, 2, \ldots, n, \ldots\} = \mathcal{Z}_+$ [11].

For the derivation of $C_X(\tau)$ (Eqn. 1) we first calculate $E(X(t))$. Using Eqn. 2 and the properties of the indicator variable described above one finds:

$$E(X(t)) = \sum_{n=1}^{\infty} E(Z(t) \cdot \gamma(n) \cdot l_{\{N(t)=n\}}) \tag{4}$$

Since the pulse heights are assumed to be independent on the pulse length, it follows from Eqn. 4 that:

$$E(X(t)) = \sum_{n=1}^{\infty} E(Z(t) \cdot l_{\{N(t)=n\}}) \cdot E(\gamma(n)) \tag{5}$$

Furthermore, if it is assumed that the distributions of the pore heights are indistinguishable:

$$E(\gamma(n)) = E(\gamma) \tag{6}$$

then it follows from Eqns. 5 and 6 that:

$$E(X(t)) = E(Z(t)) \cdot E(\gamma)$$

For the further derivation of $C_X(\tau)$ we have to determine $E(X(t) \cdot X(t+\tau))$. For this we take into account only the non-zero contributions to the right-hand

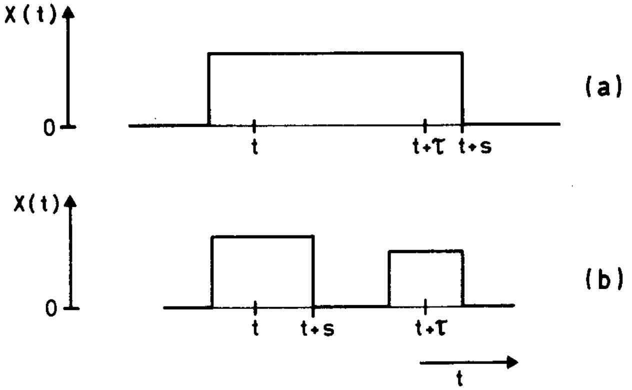

Fig. 2. Sample function $X(t)$ of the two possible cases that $X(t)$ and $X(t + \tau)$ are different from zero. s denotes the residual lifetime of a pulse already existing at time t.

side of Eqn. 1, where neither $X(t)$ nor $X(t + \tau)$ is of zero value. From Fig. 2a and b it is easily verified that since the covariance function is only a function of τ, we have to distinguish between only two different outcomes of the experiment at times t and $t + \tau$. These different outcomes can be discriminated by an additional time variable, s, called the residual life-time of an open pore. One possible outcome is that a pore open at time t, which is described by $l_{\{Z(t)=1\}}$, stays open during the time interval $t + \tau$ (Fig. 2a). This behavior of a pore can be described by the introduction of a further indicator variable $l_{\{s>\tau\}}$. The other realization which has to be taken into account is that a pore open at time t' closes during the time interval $t + \tau$ and another pore is present at $t + \tau$ (Fig. 2b). This latter behavior can be described by the random variable $l_{\{s\leqslant\tau\}}$. Therefore, $E(X(t) \cdot X(t + \tau))$ can be written as:

$$E(X(t) \cdot X(t+\tau)) = E(X(t) \cdot X(t+\tau) \cdot l_{\{Z(t)=1\}}) + \\ + E(X(t) \cdot X(t+\tau) \cdot l_{\{s\leqslant\tau\}} \cdot l_{\{Z(t)=1\}}) \tag{7}$$

Using the well-known relationship between the expectation value of the indicator variable and the probability distribution of an event, e.g., for $\{Z(t) = 1\}$ [11]:

$$E(l_{\{Z(t)=1\}}) = P\{Z(t) = 1\} \tag{8}$$

one finds from Eqn. 7:

$$E(X(t) \cdot X(t+\tau)) = P\{s > \tau\} \cdot E(\gamma^2) \cdot P\{Z(t) = 1\} + \\ + E(\gamma)^2 (E(Z(t) \cdot Z(t+\tau)) - P\{s > \tau\} \cdot P\{Z(t) = 1\}) \tag{9}$$

where we have used the familiar relationship:

$$P\{s > \tau\} + P\{s \leqslant \tau\} = 1 \tag{10}$$

Applying the definition of covariance (Eqn. 1) and of variance given by:

$$\sigma_\gamma^2 = E(\gamma^2) - E(\gamma)^2 \tag{11}$$

Eqn. 9 can be written as:

$$C_X(\tau) = P\{s > \tau\} \cdot \sigma_\gamma^2 \cdot P\{Z(t) = 1\} + C_Z(\tau) \cdot E(\gamma)^2 \quad (12)$$

Since the life-times of the pores are exponentially distributed with parameter λ, the corresponding residual life-times are also exponentially distributed with the same parameter λ, which yields [12]:

$$P\{s > \tau\} = e^{-\lambda\tau} \quad (13)$$

Furthermore, the mean of the random variable $Z(t)$ of Eqn. 3 is given by:

$$E(Z(t)) = 0 \cdot P\{Z(t) = 0\} + 1 \cdot P\{Z(t) = 1\} \quad (14)$$

where the relationship holds:

$$P\{Z(t) = 1\} = \frac{\mu}{\lambda + \mu} \quad (15)$$

For the covariance of $Z(t)$ one finds [12] that:

$$C_Z(\tau) = \frac{\lambda \cdot \mu}{(\lambda + \mu)^2} \cdot \exp(-(\lambda + \mu)\,\tau) \quad (16)$$

Using Eqns. 4 and 13—16, we obtain for $C_X(\tau)$ (Eqn. 1):

$$C_X(\tau) = \frac{\mu}{\lambda + \mu} \cdot \sigma_\gamma^2 \cdot e^{-\lambda\tau} + \frac{\lambda \cdot \mu}{(\lambda + \mu)^2} \cdot E(\gamma)^2 \cdot e^{-(\lambda+\mu)\tau} \quad (17)$$

In the following we want to compare the covariance function $C_x(\tau)$ (Eqn. 17) of the pulse sequence of conductance fluctuations shown in Fig. 1 with the known covariance function obtained for the same pulse sequence but of identical height (see, for example, Ref. 3). In particular, we are interested in the determination of the mean single-pore conductance from measurements of $C_x(\tau)$. For this comparison we introduce the probability, p, to find a pore in the open state:

$$p = \frac{\mu}{\lambda + \mu} \quad (18)$$

For determination of the mean single-pore conductance, we divide $C_x(\tau)$ by the mean of $X(t)$ which is given by the relationship:

$$E(X(t)) = p \cdot E(\gamma) \quad (19)$$

and obtain the ratio (Eqns. 17—20):

$$\frac{C_X(\tau)}{E(X(t))} = \frac{\sigma_\gamma^2}{E(\gamma)} \cdot e^{-\lambda\tau} + (1 - p) \cdot E(\gamma) \cdot e^{-(\lambda+\mu)\,\tau} \quad (20)$$

If there is a low probability of pore opening: $p << 1$ which is equivalent to $\mu \to 0$ (Eqn. 18), then Eqn. 20 has as its simplest form:

$$\frac{C_X(\tau)}{E(X(t))} = E(\gamma) \left(1 + \frac{\sigma_\gamma^2}{E(\gamma)^2}\right) \cdot e^{-\lambda\tau} \quad (21)$$

Discussion

In the preceding section, we derived the covariance function of random conductance fluctuations generated by transitions of a pore between the closed and open states (Eqn. 20), whereby in each of these two states the pore adopts only one fixed configuration. Furthermore, is was assumed that the corresponding single-pore conductance, γ, may take values between zero and infinity. Previously, it has always been assumed that a pore switching between the open and closed states may, in the open state at all times, adopt the identical conductance amplitude (see, for example, Ref. 3). This case may easily be obtained from Eqn. 20, introducing for the variance of γ: $\sigma_\gamma^2 = 0$. But single-pore experiments on planar lipid bilayer membranes using artificial pore-forming substances like gramicidin show that the variance of the single-pore conductance is different from zero [8,9]. Several explanations for this observation are discussed [9]. For example, there may be not a single form of the pore, but a range of different structures, and one of these structures may be frozen-in, when the pore forms.

Despite the fact that Eqn. 20 was derived on the basis of a unimolecular pore-forming reaction, it can also be applied for the bimolecular reaction of gramicidin-induced pore formation, since for the single-pore experiments, the number of conducting pore-dimers was small compared to the total number of gramidicin monomers in the membrane. For this example of gramicidin-induced conductance fluctuations, the assumption of $\sigma_\gamma^2 = 0$ is in contrast with the experimental finding. As application of Eqn. 21 to the measured distribution of single-pore amplitudes shows (see, for example, Fig. 2 in Ref. 8, Fig. 2 and Tables I and V in Ref. 9), an overestimation of the mean single-pore conductance $E(\gamma)$ of up to 15% must be expected for a corresponding noise analysis. Furthermore, it can be seen from Eqn. 20 or 21 that the value of $E(\gamma)$ depends not on the special shape of the amplitude distribution in the single-pore conductances, but only on the value in the corresponding variance.

It should be kept in mind that Eqn. 21 was obtained under the assumption of a low probability of pore opening ($p << 1$). As a comparison of Eqns. 20 and 21 shows, with increasing probability p an increasing overestimation of $E(\gamma)$ results from noise analysis. Furthermore it was assumed for the derivation of Eqn. 20 that the pores do not interact, therefore $E(\gamma)$ can be calculated from this relationship even in a multi-pore system.

Further contributions of the so-called intrapore noise [2] like thermal, shot of $1/f$ noise [13] were not considered for the derivation of the covariance function given in Eqn. 20.

For pores in biological membranes, such as sodium- and potassium-conducting pores in the nerve membrane, a probability distribution of single-pore conductances has not been taken into account for a determination of $E(\gamma)$ from noise analysis. But as Eqn. 20 shows, the reported values of $E(\gamma_{Na})$ and $E(\gamma_K)$ derived from noise analysis (see, for example, Ref. 5) may be systematically overestimated.

It is interesting to note that in the limit of $t = 0$, Eqn. 21 is formally identical with a corresponding expression derived for another mechanism of pore-induced conductance fluctuations as was observed on bilayer membranes in the presence of alamethicin [14—16]. Eisenberg et al. [14] analyzed fluctuations of alamethicin-doped membranes on the assumption that the membrane conductance is given as the product of two random variables, which are the number of alamethicin-induced pores and the conductance per pore. Despite the formal agreement in both cases given by the expression of Eqn. 21 at $t = 0$, which relates the variance in membrane conductance of a pore-doped membrane to the properties of the single-pore, the meaning of σ_γ^2 depends on the particular mechanism of pore-induced conductance fluctuations. In the case of alamethicin-induced conductance fluctuations, σ_γ^2 is the variance in the conductance of a single pore resulting from switching between different conductance levels while the pore is open, whereas in the case under consideration the pore can adopt only one fixed conductance level in the open position. Within the noise analysis the difference in these two mechanisms of pore conductance appears in the different time-dependence of the corresponding covariance functions.

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